Published in final edited form as:
Am Heart J. 2010 June ; 159(6): 1020–1025. doi:10.1016/j.ahj.2010.03.001.

Heart Rate is Associated with Red Blood Cell Fatty Acid Concentration: the GOCADAN Study

Sven O.E. Ebbesson, PhD[1], **Richard B. Devereux, MD**[2], **Shelley Cole, PhD**[3], **Lars O.E. Ebbesson, PhD**[4], **Richard R. Fabsitz, PhD**[5], **Karin Haack, PhD**[3], **William S. Harris, PhD**[6], **Wm. James Howard, MD**[7], **Sandra Laston, PhD**[3], **Juan Carlos Lopez-Alvarenga, MD, PhD**[3], **Jean W. MacCluer, PhD**[3], **Peter M. Okin, MD**[2], **M. Elizabeth Tejero, PhD**[3], **V. Saroja Voruganti, PhD**[3], **Charlotte R. Wenger, BA**[3], **Barbara V. Howard, PhD**[8], and **Anthony G. Comuzzie, PhD**[3]

[1] Norton Sound Health Corporation, Nome, AK, USA

[2] Weill Cornell Medical College, New York, NY, USA

[3] Southwest Foundation for Biomedical Research, San Antonio, TX, USA

[4] Uni Research AS, Bergen, Norway

[5] National Heart, Lung, and Blood Institute, Bethesda, MD, USA

[6] Cardiovascular Health Research Center, Sanford Research/University of South Dakota, Sioux Falls, SD, USA

[7] Washington Hospital Center, Washington, DC, USA

[8] MedStar Research Institute, Hyattsville, MD, USA

Abstract

Background—Consumption of omega-3 fatty acids (FAs) is associated with a reduction in deaths from coronary heart disease, arrhythmia, and sudden death. Although these FAs were originally thought to be anti-atherosclerotic, recent evidence suggests that their benefits are related to reducing risk for ventricular arrhythmia, and that this may be mediated by a slowed heart rate (HR).

Methods—The study was conducted in Alaskan Eskimos participating in the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) Study, a population experiencing a dietary shift from unsaturated to saturated fats. We compared HR with red blood cell (RBC) FA content in 316 men and 391 women ages 35–74 years.

Results—Multivariate linear regression analyses of individual FAs with HR as the dependent variable and specific FAs as covariates revealed negative associations between HR and docosahexaenoic acid (DHA; 22:6n-3; p= 0.004) and eicosapentaenoic acid (EPA; 20:5n-3; p=0.009) and positive associations between HR and palmitoleic acid (16:1n-7; p=0.021), eicosenoic acid (20:1n9; p=0.007), and dihomo-gamma-linolenic acid (DGLA; 20:3n-6; p=0.021). Factor analysis revealed that the omega-3 FAs were negatively associated with HR (p=0.003), while a cluster of other, non-omega-3 unsaturated FAs (16:1, 20:1, and 20:3) was positively associated.

*Corresponding author: Sven O E Ebbesson, PhD, GOCADAN Department, Norton Sound Health Corporation, P O Box 966, Nome, Alaska 99762, Tel: 907-479-0440, Fax: 907-452-7783, ffsoe@uaf.edu.

Conclusions—Marine omega 3 FAs are associated with lower HR, whereas palmitoleic and DGLA, previously identified as associated with saturated FA consumption and directly related to cardiovascular mortality, are associated with higher HR. These relations may at least partially explain the relations between omega-3 FAs, ventricular arrhythmia, and sudden death.

The association between consumption of long chain (C20-22) ω-3 fatty acids (FAs) in fish oil and low prevalence of coronary heart disease (CHD) was first seen in research with Greenlandic Eskimos.[1,2] Since then, additional studies have revealed a negative association between CHD mortality and consumption of fish oil.[3,4,5,6] It was originally thought that fish oil prevented atherosclerosis and/or thrombosis,[1,2] but these mechanisms (at least in Eskimo populations) have recently been questioned because even high intakes of omega-3 FA have not been associated with reduced prevalence of carotid plaque[7] or overt CHD.[8] The lower death rate with fish oil consumption is now hypothesized to be mediated by mechanisms involving the prevention of arrhythmia[9] and by increased plaque stability.[10] Elucidation of the mechanisms involved in arrhythmia is possible through studies of Eskimos, a population currently undergoing an acculturation involving a dietary shift from healthy FAs from fish oil to large amounts of non-traditional, usually saturated fats.[11] Thus, this population allows for an examination of the metabolic effects of such change on cardiovascular health.[7,8,11,12,13,14,15] In the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) Study, CHD risk factors have been examined in a genetically and culturally homogeneous population of Eskimos living in isolated villages but consuming a spectrum of traditional and store-bought foods.[12,16,17] This dietary heterogeneity lends itself to identification of associations between consumption of specific FAs and alterations in phenotypic and pathological variables.[13,14,15]

Traditional Eskimo foods, still consumed by many, contain large amounts of FA from fish oil (C20-C22 ω-3 fatty acids).[11,12,18] Mean consumption of long-chain ω-3 fatty acids in the GOCADAN cohort is 2.9 g/d compared with 0.2 g/d in the overall U.S. population. This high consumption has been associated with an improved risk factor profile for cardiovascular disease (CVD), such as a low prevalence of diabetes,[11,13,14,15] hypertension,[14,15] hypertriglyceridemia,[14,15,19] and hyperinsulinemia.[13,14,15]

In this article, the associations of FAs from fish oil and other sources with heart rate (HR), a physiological factor known to be associated with increased risk of arrhythmias and sudden death, are reported.

METHODS

Study Population

A total of 1214 predominantly Inupiat Eskimos (men and women) >17 years of age from nine villages in the Norton Sound Region of Alaska were examined in 2000–2004 for CVD and associated risk factors as part of the GOCADAN study.[16] In seven of the nine villages, an average of 82.6% of eligible residents participated.[17] Screenings were terminated early in one village, when it was determined that many villagers were away for fishing season, and in Nome, when the study reached its total recruitment goal. Of the 1214 participants, those ages 35–74 years with HR and red blood cell (RBC) FA measurements (316 men and 391 women) were included in the present analysis to allow comparison with the same age group as previous studies.[7,14] Another reason for restricting our analysis to this age group was that many of those older than age 74 years were in poor health and taking medications. The GOCADAN study was approved by the Research Ethics Review Board of the Norton Sound Health Corporation and the Institutional Review Boards of MedStar Research Institute. Informed consent was obtained from all participants. The GOCADAN study is registered at www.clinicaltrials.gov (NCT00006192).

Study Examination

The GOCADAN exam[16] consisted of a personal interview, including medical history and medication use; a physical examination, including ultrasound assessment of atherosclerosis in the carotid arteries, a Rose questionnaire, and blood sampling. A nutritional interview was conducted using a validated food frequency questionnaire.[12] Energy expenditure was estimated by metabolic equivalents (METS).

Blood pressure (BP) measurements

Following a 5-minute rest, sitting BP was measured on the right brachial artery three times with a Baum mercury sphygmomanometer (W. A. Baum, Copiague, NY).[16] The mean of the second and third measurements was used for the analysis.

Heart Rate measurement

HR was measured via computerized ECG (GE Systems MAC 1200 electrocardiograph), with the participant at rest. HR was calculated using the mean interval between QRS complexes during a 10-second period.

RBC FA composition

RBCs were obtained from EDTA blood samples after removal of the plasma and buffy coat and stored at −70°C until thawed for analysis as previously described.[20] Briefly, an RBC aliquot was heated at 100°C for 10 minutes with methanol containing 14% boron trifluoride. The FA methyl esters thus generated were extracted with hexane and water and were analyzed via gas chromatography using a GC2010 (Shimadzu Corporation, Columbia, MD) equipped with a 100m capillary column (SP-2560, Supelco, Bellefonte, PA). FAs were identified through comparison with a standard FA methyl ester mixture (GLC-727, Nuchek Prep, Elysian, MN). The coefficient of variation for very low abundance FAs (<1.0% of total FAs) was 19%; for low abundance FAs (1–10%), 6%; and for high abundance FAs (>10%), 5%.

Statistical Analysis

Data are presented as mean ± standard deviation. Variable distribution was tested for normality by Kolgomorov-Smirnov test. Variables with right-skewed distributions were transformed using a natural logarithm. In analyses for individual FAs, multivariate linear regression analysis was used with HR as the dependent variable and with consideration of other covariates significantly associated with HR. Relative RBC concentrations of specific FAs (14:0-22.6) were used as predictors of HR, with other variables significantly related to HR considered as additional covariates. Two-tailed $p<0.05$ was considered significant *a priori*. All statistical analyses were conducted in SPSS (version 12.0, SPSS, Inc., Chicago, IL).

A principal components analysis was undertaken to identify groups of FAs associated with the primary outcome measures. The extracted loadings were varimax rotated after normalization by the Kaiser method. With this method, five factors that extracted 83.5% of the total variance were identified. A multiple linear regression using the step-wise backward method was used to determine the best model for association with HR. The base model included sex, age, body mass index (BMI), diabetes status, smoking, systolic and diastolic BP, and METs as a measure of energy expenditure. The criterion for dropping a variable was $p \geq 0.10$.[10]

This study was supported by grants RO1-HL64244, UO1 HL082458, and M10RR0047-34 (GCRC) from the National Heart, Lung, and Blood Institute, Bethesda, MD. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and revising of this manuscript and its final contents. We acknowledge Rachel Schaperow, MedStar Research Institute, for editing the manuscript.

RESULTS

Participant Characteristics

On average, the population was overweight (mean BMI = 27.8), had relatively low prevalence of diabetes (6.0%), low low-density lipoprotein cholesterol (LDL-C) (123 mg/dL) and triglycerides (116 mg/dL), and high high-density lipoprotein cholesterol (HDL-C) (60 mg/dL) (Table I). Smoking prevalence (59%) was almost three times that of the general U.S. population. Carotid plaque, defined as present or absent, was detected in approximately half of the participants.[7] Beta-blockers and heart-rate slowing calcium channel blockers were taken by 56 (8%) and 13 (2%), respectively, of participants.

HR

In univariate analyses, HR was higher in women than men (74±11 vs. 71±13 bpm, p=0.003), in current smokers vs. former/never smokers (74±12 vs. 71±11 bpm, p=0.023), was correlated negatively with body height (r= 0.164, p<0.001) and positively with percent body fat as measured by bioelectric impedance (r=0.075, p=0.046) and diastolic BP (r=0.120, p=0.001). In parallel analyses, HR showed modest positive associations with BMI (r=0.075, p=0.046) and waist/hip ratio (r=0.097, p=0.011). No correlations were observed between HR and age, body weight, systolic BP, or measures of physical activity as assessed by METS.

Individual FAs and HR

In multiple linear regression analyses of individual ω-3 FAs that considered gender, height, BMI, diastolic BP, current smoking, and heart-rate slowing medications as covariates, docosahexaenoic acid (DHA; 22:6n-3) was associated with lower HR (-0.60 bpm per % of total FAs, p = 0.004), independent of significant associations with shorter height (−0.72 bpm per inch, p<0.001), current smoking (+1.8 bpm, p=0.048), higher diastolic BP (+0.15 bpm per mm Hg, p=0.001), and use of beta-blockers (−6.3 bpm, p=<0.001). In a parallel model (Table II), eicosapentaenoic acid (EPA; 20:5n-3) was associated with lower HR (−0.70 bpm per % of total FAs, p=0.007), independent of significant associations with shorter height (−0.74 bpm per inch, p<0.001), higher diastolic BP (+0.15 bpm per mm Hg, p=0.002), current smoking (+2.1 bpm, p=0.025), and use of beta-blockers (−6.4 bpm, p<0.001).

No relation was observed between HR and alpha-linolenic acid (ALA; 18:3n-3, p=0.98), independent of significant associations with shorter height, higher diastolic BP, use of beta-blockers (all p<0.001), and current smoking (p=0.016). However, a positive relation was observed between HR and palmitoleic acid (16:1n-7, +3.0 bpm per % of total FAs, p=0.002), independent of significant associations with shorter height, higher diastolic BP, beta-blocker use (all p ≤ 0.001), and current smoking (+2.1 bpm, p=0.018). Positive relations also were observed between HR and eicosenoic acid (20:1n-9; +16.2 bpm per % of total FAs, p=0.009), independent of significant associations with shorter height and beta-blocker use (both p <0.001), higher diastolic BP (p=0.002), current smoking (+2.1 bpm, p=0.023), and dihomo-gamma-linolenic acid (DGLA; 20:3n-6; +3.8 bpm per % of total FAs, p<0.002) independent of significant associations with shorter height and beta-blocker use (both p<0.001), higher diastolic BP (p=0.013), and current smoking (+2.1 bpm, p=0.019).

Factor Analysis of FAs and HR

Factor analysis (including 14 FAs) revealed five FA clusters or factors that explained 83.5% of the total FA-related variance (Tables III and IV). Factor 1 was the most strongly associated with HR. This factor included mainly three 3-omega FAs [20:5n-3, 22:5n-3, and 22:6n-3] plus lesser significant effects of three additional FAs [18:1n-9, 20:1n-9, and 20:3n-6]. The linear

combination of omega-3 FAs had a significant negative relationship with HR. Although factor 1 included three non-omega-3 FAs, the main contribution was made by the omega-3 FAs.

Relation of Dietary and RBC Omega-3 FAs

A highly significant relation was observed between RBC membrane ω-3 FA relative concentrations and dietary ω-3 FA intake (partial correlation between factor 1 and total dietary omega 3=0.39, $p<0.001$ adjusted for sex, age, BMI, and smoking). However, the reported dietary ω-3 FA intake explained less than 16% of the total variation of ω-3 FAs found in the RBC membrane.

DISCUSSION

A recent report from the GOCADAN study found no association between fish oil consumption and prevention of arterial plaque,[7] suggesting that the lower cardiovascular death rate associated with higher omega-3 fatty acid intake in epidemiologic studies and treatment trials may not be related to slowed growth of arterial plaque but to other, as yet poorly understood, effects of fish oil. Studies have shown that fish oil consumption is negatively associated with sudden death and arrhythmia,[3,4,5,6] events which are themselves associated with a higher HR.[3,4] Consequently, reports of a negative association between fish oil consumption and HR[20,21,22,23] have led to the hypothesis that the fish oil effect on HR (as well as on HR variability)[9,23,24] may contribute to the lower death rate seen with higher fish oil consumption.

In this study, we have extended understanding of this topic by examining the associations between HR and RBC membrane content of FAs in a genetically and culturally homogeneous population of Eskimos, a group which has shifted from a traditional diet rich in fish oils and monounsaturated FAs to store-bought foods rich in saturated FAs and sugar.[11,12,19] The genetic homogeneity allows for a clearer evaluation of the effects of dietary changes on pathology than is typically possible in cross-sectional studies. This article reports for the first time that not only are the RBC levels of DHA and EPA negatively associated with HR, but also that the RBC content of several saturated FAs have positive associations with HR.

Fish Oil and HR

The data show a significant negative association between HR and a biomarker of omega-3 FA intake, RBC EPA and DHA levels. The average consumption of fish oil ω-3 FAs in this population is about 2.9 g/d compared with 0.2 g/d in the general U.S. population.[7,12] These results agree with other large cross-sectional studies linking fish intake with HR (Mozaffarian et al.[22] [n=5,096]; Chrysohoou et al.[23] [n=3,042]; Dallongeville et al.[25] [n=9,758]) and with intervention studies that show a negative association between fish oil and HR.[25,26,27] In some studies, the relations were stronger for DHA than for EPA,[25,28] and the effects of fish oil supplementation ranged from −2 to −5.8 beats/minute.

The mechanism responsible for the inverse relation between HR and ω-3 FAs (whether by biomarker or intake) is not known. It does not appear to require vagal innervation,[29] but in subjects with normal vagal tone, enhanced parasympathetic activity may play a role. Although Mori et al. reported that supplemental DHA lowered HR while EPA did not, we found a significant association between both long chain ω-3 FAs and HR in this population. Although there is evidence that EPA may not be incorporated into the cardiac phospholipids,[30] the current data suggest that the mechanism by which ω-3 FAs affect HR are mediated via the incorporation of these FAs into the heart muscle itself. One hypothesis emerging from these data is that the association between fish oil and HR results indirectly from greater cardiac efficiency, produced by the lower diastolic BP associated with fish oil ω-3 FAs, which may contribute to a slower

heartbeat. Our data show for the first time in an epidemiological study a significant association between diastolic BP and DHA ($p<0.001$).

The lower diastolic BP associated with fish consumption[28] is thought to result from fish oil stimulation of nitric oxide production, which increases vasodilatation of the large arteries and vessels.[15] The exact mechanism is not known, but the evidence suggests that it may involve the inhibition of thromboxane-mediated vasoconstriction. The evidence further suggests that fish oils increase the left ventricular ejection fraction by enhancing ventricular filling during diastole, thus providing an energy-sparing promotion of diastolic relaxation.[31] Fish oils also raise the electrical threshold at which ventricular fibrillation can be induced and are effective in decreasing pro-arrhythmic thromboxane.[31] Other effects of fish oil consumption include improved blood flow[29] and decreased arterial stiffness.[32]

Diastolic BP, which is low in Eskimos, is negatively associated with increased fish oil consumption in both the Alaska Siberia Project (ASP) and the GOCADAN study.[13,14,15] Significantly lower diastolic BP, but not systolic BP, has been observed in Eskimos who improved their glucose tolerance in an intervention study that resulted in decreased palmitate consumption and increased ω-3 FA consumption.[13]

Other FAs and HR

The GOCADAN data do not reveal any association between ALA (18:3-n3) and HR, although other researchers have reported a negative association.[33] The associations of HR with an omega-6 fatty acid (20:3n-6, DGLA) and with the monounsaturated FAs palmitoleic acid (16:1n-7) and eicosenoic acid [20:1n-9]) have, to our knowledge, not been reported previously. The associations between RBC concentrations of these specific FAs and HR appear to show an opposite effect to that of ω-3, suggesting a deleterious effect on HR that needs to be verified by additional research. These three FAs have been identified as potentially harmful in raising other CVD risk factors.[34] For example, palmitoleic acid is biosynthesized from palmitate by delta 9 desaturase and behaves like a saturated FA and increases cholesterol levels.[35] It is found in macadamia oil and marine mammals, is a product of endogenous lipogenesis, and correlates with indexes of adiposity. Eicosenoic acid (e.g., from peanut oil and butter), is an elongation product of oleic acid and contributes to 10% of adiponectin variance.[34] DGLA is positively associated with diastolic BP.[34]

The current findings on the associations of specific non ω-3 FAs with HR complement the recent report of a prospective study of Swedish men showing that serum FAs associated with saturated fat intake (palmitic, palmitoleic, and DGLA) were positively related to total and cardiovascular mortalilty.[35] It is also noteworthy that other FAs associated with cardiac mortality in the Swedish study tended to be positively associated with HR in the present study. These included palmitic acid (16:0; $p=0.068$), oleic acid (18:1n-9; $p=0.63$), and linoleic acid (18:2n-6; $p=0.083$). The results thus suggest that the observed cardiovascular mortality could be, at least partially, due to a dietary FA-mediated effect on HR. To what extent the non-omega-3 FA content of RBCs correlates with dietary intake is unknown;[35] therefore, defining the extent to which dietary changes can affect RBC concentrations of these FAs will require further study. Furthermore, additional research is needed to determine whether the present findings are also observed in other population-based samples with differing lifestyles and ethnicities (e.g., the Cardiovascular Health Study and Framingham Heart Study).

Conclusion

FAs derived from fish oils are associated with lower HR, which may in turn reduce risk for arrhythmia and sudden death, whereas several non-omega 3 FAs are associated with higher HR, independent of the relevant covariates. The latter FAs have previously been identified as

associated with other CVD risk factors. Palmitoleic acid (16:1n-7) and DGLA (20:3n-6) have been positively related to cardiovascular mortality, suggesting an association between cardiovascular mortality and HR that is affected by FAs.

Acknowledgments

The study was funded by grants R01-HL64244, U01 HL082458, U01 HL082490, and M10RR0047-34 (GCRC) from the National Heart, Lung, and Blood Institute of the National Institutes of Health, Bethesda, MD. The authors are grateful to the Norton Sound Health Corporation (NSHC) and the participants of villages participating in this study. We thank Rachel Schaperow, MedStar Research Institute, for editorial services.

References

1. Dyerberg J, Bang HO, Hjorne N. Fatty acid composition of the plasma lipids in Greenland Eskimos. Am J Clin Nutr 1975;28:958–966. [PubMed: 1163480]
2. Dyerberg J, Bang HO, Stoffersen E. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet 1978:117–119. [PubMed: 78322]
3. Burr ML, Gilbert JF, Holliday RM, et al. Effects of changes in fat fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 1989;ii:757–761. [PubMed: 2571009]
4. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-prevenzione trial. Lancet 1999;354:447–455. [PubMed: 10465168]
5. Marckmann P, Gronbaek M. Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. Eur J Clin Nutr 1999;53:585–590. [PubMed: 10477243]
6. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. New Engl J Med 2002;346:1113–1118. [PubMed: 11948270]
7. Ebbesson SOE, Roman MJ, Devereux RB, et al. Consumption of omega-3 fatty acids is not associated with a reduction in carotid atherosclerosis: The Genetics of Coronary Artery Disease in Alaska Natives study. Atherosclerosis 2007;199:346–353. [PubMed: 18054937]
8. Ebbesson SOE, Risica PM, Ebbesson LOE, et al. Eskimos have CHD despite high consumption of omega-3 fatty acids; The Alaska Siberia Project. Int J Circumpolar Health 2005;64:416–424.
9. Leaf A, Kang JX, Xiao YF, et al. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 2003;107:2646–2652. [PubMed: 12782616]
10. Thies F, Garry JM, Yaqoob P, et al. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. Lancet 2003;361:477–485. [PubMed: 12583947]
11. Ebbesson SO, Kennish J, Ebbesson L, et al. Diabetes is related to fatty acid imbalance in Eskimos. Int J Circumpolar Health 1999;58:108–119. [PubMed: 10429340]
12. Nobmann ED, Ponce R, Mattil C, et al. Dietary intakes vary with age among Alaskan Eskimo Adults of Northwest Alaska, in the GOCADAN Study, 2000–2003. J Nutr 2005;135:856–862. [PubMed: 15795447]
13. Ebbesson SOE, Ebbesson LOE, Swenson M, et al. A successful diabetes prevention study in Eskimos: The Alaska Siberia Project. Int J Circumpolar Health 2005;64:400–415.
14. Ebbesson SOE, Risica PM, Ebbesson LOE, et al. Omega 3 fatty acids improve glucose tolerance and components of the metabolic syndrome in Alaskan Eskimos: The Alaska Siberia Project. Int J Circumpolar Health 2005;64:365–377. [PubMed: 16277121]
15. Ebbesson SOE, Tejero ME, Nobmann ED, et al. Fatty acid consumption and metabolic syndrome components: The GOCADAN Study. J Cardiometab Syndr 2007;2:244–249. [PubMed: 18059206]
16. Howard BV, Devereux RB, Cole SA, et al. A genetic and epidemiologic study of cardiovascular disease in Alaska Natives (GOCADAN): Design and methods. Int J Circumpolar Health 2005;64:206–221. [PubMed: 16050315]

17. Ebbesson SOE, Laston S, Wenger CR, et al. Recruitment and community interactions in the GOCADAN study. Int J Circumpolar Health 2006;65:23–32.

18. Risica PM, Nobmann ED, Caulfield LE, et al. Springtime macronutrient intake of Alaska Natives of the Norton Sound area. Int J Circumpolar Health 2005;64:222–233. [PubMed: 16050316]

19. Harris WS. N-3 fatty acids and lipoproteins: comparison of results from human and animal studies. Lipids 1996;31:243–252. [PubMed: 8900453]

20. Harris WS, Lemke SL, Hansen SN, et al. Stearidonic aicid-enriched soybean oil increased the omega-3 index, an emerging cardiovascular risk marker. Lipids 2008;43:805–811. [PubMed: 18683001]

21. Fox K, Ford I, Steg PG, et al. Heart rate as a prognostic risk factor in patients with coronary artery disease and left-ventricular systolic dysfunction: a subgroup analysis of a randomized controlled trial. Lancet 2008;372:817–21. [PubMed: 18757091]

22. Mozaffarian D, Prineas RJ, Stein PK, et al. Dietary fish and n-3 fatty acid intake and cardiac electrocardiographic parameters in humans. J Am Coll Cardiol 2006;48:478–484. [PubMed: 16875972]

23. Chrysohoou C, Panaglotakos DB, Pitsavos C, et al. Long-term fish consumption is associated with protection against arrhythmia in healthy persons in a Mediterranean region – the ATTICA study. Am J Clin Nutr 2007;85:1385–1391. [PubMed: 17490977]

24. O'Keefe JH Jr, Abuissa H, Sastre A, et al. Effects of omega-3 fatty acids on resting heart rate, heart rate recovery after exercise, and heart rate variability in men with healed myocardial infarctions and depressed ejection fractions. Am J Cardiol 2006;97:1127–1130. [PubMed: 16616012]

25. Dallongeville J, Yarnell J, Ducimetlère P, et al. Fish consumption is associated with lower heart rates. Circulation 2003;108:820–825. [PubMed: 12912821]

26. Harris WS, Gonzales M, Laney N, et al. Effects of omega-3 fatty acids on heart rate in cardiac transplant recipients. Am J Cardiol 2006;98:1393–1395. [PubMed: 17134636]

27. Christensen JH. N-3 fatty acids and the risk of sudden cardiac death. Emphasis on heart rate variability. Dan Med Bull 2003;50:347–567. [PubMed: 14694851]

28. Theobald HE, Goodall AH, Sattar N, et al. Low-dose docosahexaenoic acid lowers diastolic blood pressure in middle-aged men and women. J Nutr 2007;137:973–978. [PubMed: 17374663]

29. Walser B, Giordano RM, Stebbins CL. Supplementation with omega-3 polyunsaturated fatty acids augments brachial artery dilation and blood flow during forearm contraction. Eur J Appl Physiol 2006;97:347–354. [PubMed: 16770472]

30. Grynberg A. Hypertension prevention: from nutrients to (fortified) foods to dietary patterns. Focus on fatty acids. J Hum Hypertens 2005;19(Suppl 3):S25–33. 30. [PubMed: 16302007]

31. McLennan PL, Barnden LR, Bridle TM, et al. Dietary fat modulation of left ventricular ejection fraction in the marmoset due to enhanced filling. Cardiovasc Res 1992;26:871–877. [PubMed: 1451164]

32. Sugawara J, Komine H, Hayashi K, et al. Effect of systemic nitric oxide synthase inhibition on arterial stiffness in humans. Hypertens Res 2007;30:411–415. [PubMed: 17587753]

33. Christensen JH, Schmidt EB, Mølenberg D, et al. Alpha-linolenic acid and heart rate variability in women examined for coronary artery disease. Nutr Metab Cardiovasc Dis 2005;15:345–351. [PubMed: 16216720]

34. Arnlöv J, Lind L, Sundström J, et al. Insulin resistance, dietary fat intake and blood pressure predict left ventricular diastolic function 20 years later. Nutr Metab Cardiovasc Dis 2005;15:2242–2249.

35. Warensjö E, Sundström J, Vessby B, et al. Markers of dietary fat quality and fatty acid desaturation as predictors of total and cardiovascular mortality: a population-based prospective study. Am J Clin Nutr 2008;88:203–209. [PubMed: 18614742]

Table I

Baseline Characteristics

Variable	Mean±SD or N (%)	Range
Age (years)	50±10	35 – 74
Gender (women/men)	391/316	- - - - -
Heart rate (beats/minute)	73±12	46 – 122
Systolic blood pressure (mm Hg)	120±15	84 – 174
Diastolic blood pressure (mm Hg)	77±9	50 – 114
Hypertension (JNC-7, %)	203 (29%)	- - - - - -
Body mass index (kg/m^2)	27.8±5.9	16.7 – 54.9
Diabetes	44 (6%)	- - - - - -
Smoking (%)	415 (59%)	- - - - - -
Docosahexaenoic acid (% of total FAs)	6.7±2.2	0.6 – 13.7
Eicosapentaenoic acid (% of total FAs)	2.2±1.7	0.2 – 10.2
Palmitic acid (% of total FAs)	20.9±1.8	17.8 – 35.9
Palmitoleic acid (% of total FAs)	0.8±0.5	0 – 3
Eicosenoic acid (% of total FAs)	0.20±0.07	0.05 – 0.96
Dihomo-gamma-linolenic acid (% of total FAs)	1.7±0.5	0.4 – 3.6

Table II

Heart rate associated with individual fatty acids

Fatty Acid	Effect on Heart Rate	p-value
18:3n-3 alpha-linolenic acid (ALA)		0.98
20:5n-3 eicosapentaenoic acid (EPA)	−0.70 bpm	0.007
22:6n-3 docosahexaenoic acid (DHA)	−0.60 bpm	0.004
16:1n-7 palmitoleic acid	+3.0 bpm	0.002
20:1n-9 eicosenoic acid	+16.2 bpm	0.009
20:3n-6 dihomo-gamma-linolenic acid (DGLA)	+3.8 bpm	0.021

bpm = beats per minute.

Data are from multiple linear regression analyses of individual ω-3 FAs that considered gender, height, BMI, diastolic blood pressure, current smoking, and heart-rate slowing medications.

Table III

Red blood cell fatty acid concentrations and factor loadings for varimax orthogonal five-factor solution after Kaiser normalization.

Fatty Acid	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Commonality
22:6n3	**−0.845**	−0.216	0.061	−0.145	0.157	0.810
20:5n3	**−0.819**	−0.302	0.062	−0.072	0.222	0.819
20:1n9	**0.806**	−0.046	0.033	−0.323	0.094	0.765
22:5n3	**−0.716**	−0.044	−0.057	−0.379	0.324	0.767
20:3n6	**0.651**	0.416	−0.050	0.116	−0.101	0.623
18:1	**0.532**	**−0.569**	−0.117	0.327	0.377	0.869
20:4n6	0.013	**0.934**	0.045	−0.046	0.023	0.877
22:4n6	0.389	**0.837**	0.012	−0.072	0.169	0.885
22:5n6	0.402	**0.767**	0.215	−0.052	0.061	0.803
24:1n9	−0.074	0.035	**0.976**	0.004	0.073	0.964
24:0	0.015	0.133	**0.966**	−0.062	0.005	0.956
16:1	−0.081	−0.317	−0.046	**0.852**	0.161	0.861
18:3n6	0.231	0.118	−0.024	**0.809**	−0.300	0.812
18:2n6	0.292	−0.123	−0.096	0.091	**−0.898**	0.924

Table IV

Multiple linear regression analysis for variables associated with heart rate.

	Beta	r_p	p-value
Diastolic blood pressure	0.172	0.170	<0.001
Glucose intolerant	0.10	0.102	0.020
Age	0.09	0.085	0.051
Sex	−0.18	−0.178	<0.01
Current smoking	0.10	0.96	0.028
Omega-3 (Factor 1)	0.13	0.124	0.005

Columns show the standardized beta and the partial correlation coefficients (r_p). The model dropped METs, BMI, systolic blood pressure, and factor scores 2–5. Diabetes status and smoking were treated as simple contrasts against normal glucose and never smoking.